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AN EXAMINATION OF THE CEMENTUM OF THE TEETH OF BOVIDAE WITH SPECIAL REFERENCE TO ITS USE IN AGE DETERMINATION

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate studies for acceptance, a thesis entitled An Examination of the Cementum of the Teeth of Bovidae with Special Reference to Its use in Age Determination submitted by George Gaylen Armstrong in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The complete tooth row of four species of Bovidae; Bison bison bison (bison), Ovis canadensis canadensis (bighorn sheep), Oreamnos americanus americanus (mountain goats), and Bos taurus (domestic cattle), was studied systematically to analyze the growth patterns of dental cementum of teeth.

Thick and thin sections of teeth were prepared and examined microscopically. Annuli in the tooth cementum of bison, bighorn sheep, and mountain goats (the wild species), were observed adequately in thick sections under reflected light, with alcohol applied to the cut or ground surfaces. Annuli in the tooth cementum of domestic cattle, were observed adequately in thin sections, with transmitted light. Each annulus consists of one opaque layer alternating with a translucent layer of cementum.

Thick sections of horns of bighorn ewes were prepared and examined macroscopically. The sections revealed annuli similar to those observed in the tooth cementum of the wild species. The annuli in horn sections are discussed in relationship to age.

Results from the preliminary examination of the complete tooth row of the wild species, suggested that cementum

annuli are related to age.

From the complete tooth row, the root cementum of the lower canines of bison was chosen to compare annuli with age and tooth wear. The coronal cementum of the third molars of bighorn sheep rams, was chosen to compare annuli with the number of horn rings. In bison, a significant correlation ($X^2(19) = 0.75$; $P = <0.05$) occurred between known age and annuli. Also, a significant correlation ($r = 0.851$; $P = < 0.05$) was obtained between annuli and tooth wear. In bighorn sheep rams, a significant correlation ($X^2(46) = 7.95$; $P = <0.05$) was obtained between annuli and horn rings.

In bison and bighorn sheep rams, the opaque layers of cementum were deposited from spring to fall, the translucent layers sometime during the winter months. The causative factors for this seasonal change in cementum are discussed. Also reasons are suggested for the occurrence of false annuli, "rut lines" and finer lamellae in the tooth cementum of the wild species.

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INTRODUCTION

This study is concerned with the use of tooth cementum in determining the age of some species of North American Bovidae. The major part of the study concerns some aspects of tooth growth and structure in *Bison bison bison* (bison), and *Ovis canadensis canadensis* (bighorn sheep), with minor investigations carried out on *Oreamnos americanus americanus* (mountain goats), and *Bos taurus* (domestic cattle).

In previous studies of some big game mammals, it has been assumed that annuli in the cementum of teeth are equivalent to annual increments. The main objective of this study is to critically test this assumption by studying systematically, the complete tooth row of the species concerned. To date, there does not appear to have been such a systematic study done on any mammal.

BRIEF LITERATURE REVIEW

The most reliable method of aging North American bovids up to the present time, has been by tooth replacement and wear, and horn growth. In young bison, for example, Fuller (1959) devised a method of determining the age based on the eruption of the permanent teeth. This method has been confirmed with known-age jaws by Armstrong (unpublished). Fuller (1959) had to depend on wear once the dentition was complete, and was able to recognize only three age categories in animals older than 4 years: young adult, adult, and aged. Young bighorn sheep were aged to the nearest 6 months by tooth replacement (Taber, 1963). Also, they were aged by annual horn growth to the approximate age of 8 years in rams and 5 years in ewes (Cowan, 1940). After these ages, horn growth was so slow that age determination became too subjective. This same general difficulty was experienced by Brandborg (1955) with mountain goats.

It was from work done on marine mammals that a possibility of a more accurate method of age determination of big game ungulates has come to light. Scheffer (1950) and Laws (1952, 1953), working respectively with northern fur seals (*Callorhinus ursinus cynocephalus*) and elephant seals

(*Mirounga leonina*), found that there were rings in the dentine which seemed to be formed at the rate of one per year. Since then, other marine mammals have been investigated and annuli have been found in the dentine (Fisher, 1954; Laws, 1958; McLaren, 1958; Nishiwaki *et al.*, 1958; and Hewer, 1960). Cementum was also found in the teeth of marine mammals, but usually the annual growth layers were difficult to see. Sergeant and Pimlott (1959) found growth layers in the cementum of incisor teeth of moose (*Alces alces*) with less marked periodic growth in the dentine. The cementum showed regular alternations of opaque and translucent growth zones. A full annual layer was found to consist of one opaque and one translucent zone. They stated that "it seemed probable that opaque cement is laid down in summer and fall, translucent cement in winter and spring". Later, Low and Cowan (1963) and McEwan (1963) used the first incisor to age respectively, mule deer (*Odocoileus hemionus*) and caribou (*Rangifer tarandus*). Mitchell (1963) determined the age of red deer (*Cervus elaphus*) by counting annual layers in the cementum pad of the first lower molar (M_1). Novakowski (1964) found cementum layers in the roots of the last lower premolar (PM_4) and related this to the age of bison (*Bison bison*).

This method of determining age seems promising, although

all of the studies done on these terrestrial big game ungulates, with the exception of that of Low and Cowan (1963), lacked a representative sample of known-age jaws to confirm the results.

MATERIALS AND METHODS

Preparation and Examination of Material

The material available for this study is shown in Table I.

Removal of teeth. - The complete tooth row of each ramus from two lower jaws of bison was removed for study. Also teeth chosen at random from an additional 17 lower jaws were removed for study. Each of the bison jaws was aged by tooth replacement and wear (Fuller, 1959). The complete tooth row from one ramus per jaw of two lower jaws was removed for study in bighorn sheep, mountain goats, and domestic cattle. The age of bighorn sheep and mountain goats was based on the number of horn rings. The "plus" sign, placed after a specified number of horn rings, refers to the amount of horn sheath present subsequent to the most recently formed horn ring. The domestic cattle were of known age.

The incisiform teeth and the premolars were removed by submerging the jaws in boiling water for approximately 5 min and then pulling the teeth out with a pair of dental forceps. The molars were removed by placing the ramus of the lower jaw in a vice, and chipping away the lower portion of the ramus with a cold chisel and hammer. If bone still adhered to the

Table 1. Number of jaws, area, and date of collection of the species used in this study.

Species	Location	Number of Jaws	Collection Dates
Bison	Wood Buffalo Park, Northwest Territories.	58**	Nov. 1960, 1961, and May, 1963.
	Elk Island National Park, Alberta.	13*	December, 1964.
	Wichita Mountain Wildlife Refuge, Oklahoma.	6*	December, 1964.
Bighorn Sheep	Eastern slopes of the Rocky Mountains, Alberta. (49-54° N)	34**	May to November, 1960-1964.
		15[1]	"
		5[2]	"
Mountain Goats	Pinto Creek, Alberta. (53° 51'N - 117° 35'W)	1**	July, 1963.
		1**	October, 1964.
Domestic Cattle	Holden, Alberta.	1*	August, 1964.
	Kinsella, Alberta.	1*	November, 1964.

*known-age jaws

**unknown-age jaws

[1] upper jaws

[2] pairs of matching upper and lower jaws

teeth, it was removed by additional chipping, and prying with a screwdriver. Breakage of the molars occurred, although this did not affect preparation of the teeth for sectioning. Each tooth was labelled and stored in a vial of 70% alcohol.

Sectioning of teeth. - Thin sections of the roots of all teeth were prepared by first removing the crowns of the teeth transversely with a hack saw (14 teeth per in.) or a band saw (18 teeth per in.). The anterior roots of molariform teeth of bison and cattle, with the exception of the second and third lower premolars (PM_2 and PM_3), were separated from the posterior roots by making a transverse cut below the base of the crown. This separation was required only for the third lower molar (M_3) in mountain goat and bighorn sheep teeth. The teeth were cut up in this fashion so that each piece was small enough to be mounted on the Niclas Bone and Tooth Cutting Machine (W.E. Niclas, 148-25 89th Avenue, Jamaica 35, New York) (Fig. 1 and 2). Before a tooth was sectioned, an adapter ring, which later fits into the specimen holder, (2 in Fig. 2), was lined with wax by dipping the ring in hot wax and allowing it to harden. This process was repeated. The wax was then removed from the outer portion of the adapter ring. A thin paste was made of powdered dental

rock, and water. The paste was placed in the adapter ring so that it protruded about 1/8 in. above one end of the ring. Part of the tooth to be sectioned was immediately placed in the wet paste at the angle desired for the particular plane of cut so that the tooth penetrated at least 1/8 in. into the dental rock. Large roots were anchored in the dental rock by drilling a hole 3/16 in. deep and 5/64 in. in diameter into the tooth and wedging a stick ½ in. long and 3/32 in. in diameter into the drilled hole. The tooth was then placed, stick downwards, into the dental rock. After the tooth was placed in the wet paste, the mount was allowed to dry for approximately 5 min. Excess paste was scraped off the adapter ring. The specimen was then mounted in the specimen holder (2 in Fig. 2).

The Niclas Bone and Tooth Cutting Machine consists of a stainless steel shaft (1) mounted on ball bearings. The specimen in the adapter ring is held in the specimen holder (2) attached to a stainless steel micrometer screw (4) which allows precise advancement of the specimen towards the cutter (11). The micrometer dial is graduated in thousandths of an inch. The cutter is a carborundum blade 0.010 in. thick. The machine is designed to cut down to 0.003 in. but 0.007 in. (177.8μ) was found to be satisfactory for this study.

The cutter, which rotates at 3600 rev/min, is lubricated and cooled by a continuous flow of water (6). The cutting area is enclosed by a splash guard (8), one side of which is removable to facilitate removal of the cut section. The specimen is fed to the cutting wheel by gravity and the rate of feed is controlled by a simple adjustable balance weight (12) attached to the micrometer bracket.

In this study each tooth was mounted in the specimen holder, aligned with the cutter, and the locking screw (13) tightened. The micrometer lock screw (3) was loosened and the micrometer rotated until the line of cut was passing near the middle of the root. Water flow was adjusted to a slow and steady drip. The motor was turned on and the specimen was slowly advanced, by hand, until it engaged the carborundum cutting disk, when the gravity feed was allowed to take over. Just before the cut was complete the advance of the specimen was stopped, and the section was broken off manually, which saved searching for it in the bottom of the trough. Poor wedge-shaped sections are made if the tooth is allowed to advance toward the cutting blade too rapidly or if the blade is worn excessively.

Longitudinal and transverse serial sections were made on the roots of the incisiform and molariform teeth.

Longitudinal sections were cut either parallel with or perpendicular to the longitudinal axis of the molariform teeth.

Two or three sections were taken from each specimen and replaced in their original vial of 70% alcohol. The dental rock and the embedded tooth was removed from the adapter ring by heating the ring, which melted the wax lining.

After thin sections were removed by the cutting machine, the remaining part of the tooth acted as a thick section. This was the only way thick sections were prepared for teeth of domestic cattle and mountain goats. Other thick sections, however, were prepared in three additional ways for the teeth of bison and bighorn sheep:

(a) Roots to be sectioned were cut with a band saw or ground with a power sander using no. 80 coarse sandpaper. The ground or cut surfaces were then polished by hand with different grades of sandpaper (silicon carbide) in the following order: 180C; 240C; 400A; and 600A. The polished surfaces were examined at intervals and the grinding was continued until the maximum number of cementum layers appeared.

(b) Thick sections of the coronal cementum of the molars were prepared with the molars still in the ramus, by making a transverse cut of the tooth with a band saw just below the alveolar border of the ramus. However, in bighorn

sheep teeth, the exposed coronal cementum was coated with a white glue,"Wender Ev-A-Grip", and allowed to dry overnight before sectioning. This practice reduced the incidence of breakage or loss of the thin coronal cementum when cut with a band saw. Both cut surfaces of the teeth were polished with a series of sandpapers as described above.

(c) The lingual cementum (cementum on the tongue-side of the tooth crowns) is thickest between the anterior and posterior cusps of the molars. It was removed from molars of bison that were still present in the jaw by freeing the dry gum and periodontal connective tissue away from the tooth with a scalpel or dissecting needle, and then inserting a dissecting needle between the cementum and enamel at the occlusal surface of the tooth. The periodontal connective tissue is found between the alveolus and the tooth. Care was taken to insure that the lingual cementum broke off transversely and well below the alveolar border. The position of the alveolar border was marked with a pencil on the lingual cementum before its removal. The broken surface of the lingual cementum was then sanded and polished up to the pencil mark, with a series of sandpapers as described above. Labial cementum (cementum on the cheek-side of the tooth crowns) was not removed in the above fashion because of the presence of a style

on the labial side of the crown.

The lingual cementum cannot be removed, as described above, from teeth of freshly killed bison because the cementum is still firmly united with the enamel and it is thus impossible to separate the two tissues.

Mounting of tooth sections. - Thin sections of bison teeth were mounted as follows:

(a) Nitrocellulose and Canada balsam.

Sections were cleaned in distilled water for 1 min and then placed in nitrocellulose for a few seconds to prevent the penetration of Canada balsam into the tooth tissue. The sections were then mounted in Canada balsam.

(b) Xylene and Canada balsam.

Sections were washed in distilled water for 1 min, brought up through alcohol, placed in xylene for 1 min, and then mounted in Canada balsam.

(c) Erlich's haematoxylin, Canada balsam, and Aquamount.

Sections were placed in distilled water for 5 min and then placed in Erlich's haematoxylin for 20 min. The sections were then washed in tap water for 15 min. The process was repeated if the sections were understained. Overstained sections were destained in 1% HCl and distilled water.

Sections were mounted in Aquamount or brought up through alcohol to xylene and mounted in Canada balsam.

(d) Aquamount.

Sections were washed in distilled water and then mounted directly in Aquamount. Some sections were placed in a 50% solution of Aquamount and distilled water under pressure for 10 min and then mounted on slides.

(e) Alcohol or no mounting medium.

Sections were stored in their vials of 70% alcohol until viewed microscopically either moistened with a drop of 70% alcohol on the polished surface, or left dry.

Thick sections of bison teeth were prepared for examination either as in (e) above or etched by decalcification (molars only). Three different etching techniques were used:

(a) Sections were submerged in 100 ml. of 10% or 30% hydrochloric acid for 10 hr and allowed to dry before examination.

(b) Sections were submerged in 10%, 20%, or 30% acetic acid for 20 hr, then washed and allowed to dry. Sections placed in 10% acetic acid were washed at the second and fourth hours of the 20-hr period.

(c) Sections were submerged in a solution of 5% hydrochloric acid and 95% ethyl alcohol for 2 hr, washed and

allowed to dry overnight (Novakowski, 1964).

The cementum layers were adequately seen with alcohol in thin and thick sections of bison teeth. No improvement was made in the clarity of the layers by placing thin sections in other media or thick sections in decalcifying solutions. On this basis, thin and thick tooth sections of bighorn sheep, mountain goats, and domestic cattle were mounted as outlined on p. 13, paragraph (e).

Microscopic examination of tooth sections. - Thin sections of teeth were examined in transmitted, reflected, and polarized light with total magnifications of 10X, 20X, 40X, 100X, and 400X. Thick sections of teeth were examined in reflected light (light striking the cut or ground surface of the tooth section) at 10X, 20X, and 40X magnifications. Thick sections of all teeth were rotated in reflected light so that the greatest contrast in cementum layers was achieved.

For purposes of this study the cementum layers were seen adequately in thick tooth sections with reflected light at 20X, and 40X magnifications in bison, bighorn sheep, and mountain goats. Cementum layers in cattle teeth were best seen in thin tooth sections in transmitted light with a magnification of 40X.

Thin sections in transmitted light with 100X and 400X magnifications showed more cellular detail, but at the same time, obscured the cementum layers. Polarized light did not improve the clarity of the layers or cellular detail of cementum, significantly.

The number of cementum layers in teeth of the complete tooth row of each species was observed and recorded.

Sectioning of horns. - A cut, anteroposterior to the longitudinal axis of the horn, was made with a band saw, the entire length of each horn from six ewes. The cut was made slightly off the midline. This was to ensure that the full complement of internal horn rings could be observed on the thicker portion. The surfaces were sanded with no. 80C, 100C, 150A, and 220A sandpaper, in that order. The surfaces were then moistened with water and observations were made.

Statistical Analysis

The Chi-square (X^2) test, at the 95% confidence level, was used to determine the significance of expected and observed frequencies. The coefficient of correlation was calculated to determine if an association existed between canine wear and the number of annuli in the canine cementum of bison. When this proved to be high a linear regression was done.

The reference used for statistical analyses was Steel and Torrie (1960).

RESULTS

Examination of Material

Examination of the complete tooth row. - Each tooth sits in an alveolus. The alveolus, made up of porous bone, fits the shape of the tooth present (Fig. 3).

Thick sections of teeth show that the cementum covers the entire surface of the roots, thinning out at the cemento-enamel junction. Cementum is deposited along the contours of multirooted teeth. In incisiform teeth the cementum takes the form of a cone-within-a-cone construction about the tooth roots. Local thickenings of root cementum occur in the bifurcation of multirooted teeth and on the posterior concave (lingual) surfaces of incisiform roots. The greatest number of cementum layers occurs at these local thickenings. The thick cementum at the bifurcation of multirooted teeth has been referred to as a cementum pad (Fig. 4). The cementum is proportionally thicker at the root apices than it is at the bifurcations of multirooted teeth in bighorn sheep and mountain goats (Fig. 5), compared to the multirooted teeth of bison and cattle.

The cementum continues to cover the entire lingual and labial surfaces of the crowns of the molars (Fig. 3). The

premolars have less coronal cementum both in thickness and area of coverage. The coronal cementum of the molariform teeth of mountain goats is practically absent, and is too thin to section even in the oldest specimens available (Table 2).

Layers occur in both dentine and cementum of all teeth. The dentine layers in transmitted light consist of alternating light and dark bands. The boundaries of the dentinal growth layers are usually indistinct when compared to those of cementum layers. Cementum can be seen in Fig. 6. It contains layers with distinct boundaries, and therefore, it is used as the criterion for aging in this study. These layers consist of alternating opaque and translucent cementum. The opaque layers consist of greater concentrations of cementocyte cells than is found in the translucent layers. Also, in bison teeth, a decalcifying solution produces an etching effect by dissolving away proportionally more of the opaque cementum.

One opaque and one translucent layer of cementum, in that order, is considered to be one annulus. When the outermost layer, found in a specimen, was opaque, it was recorded as half an annulus. In all the tooth sections, the cementum layer next to the dentine in tooth roots and next to the enamel in tooth crowns, is opaque. Also, the outermost layer,

Table 2. The maximum number of annuli in the cementum of the teeth of the complete tooth row in bison, bighorn sheep, mountain goats, and domestic cattle. The teeth are listed in the approximate order of their appearance in the jaw.

Species	Age and Sex	Ramus	M_1 ante-rior root	M_1 poste-rior root	M_1 cemen-tum pad	M_1 crown	M_2 ante-rior root	M_2 poste-rior root	M_2 cemen-tum pad	M_2 crown	I_1 root	I_2 root	PM_2 roots	PM_3 roots	PM_4 ante-rior root	PM_4 poste-rior root	PM_4 cemen-tum pad	I_3 root	M_3 ante-rior root	M_3 poste-rior root	M_3 cemen-tum pad	C_1 crown	C_1 root
bison	Young adult male (no. 751)	left	4½	4½	4½	5½-6½	3½	3½-4½	3½	3½-4½	3½-4½	2½	4½	2½	1½	1½	broken	2½-3½	½-1½	1½	X	4½	1½
		right	X	1½	5½	4½-5½	4½	2½	3½	broken	3½	1½	3½-4½	½-1½	broken	broken	broken	1½	½	½	½	3½	1½
	Aged female (no. 741)	left	15½-16½	16½	14½-15½	14½-15½	X	13½-14½	13½-14½	broken	15½-16½	16½	15½-16½	broken	13½-14½	13½-14½	13½-14½	11½	13½	13½-14½	13½-15½	15½	13½
		right	16½	16½	16½-17½	13½	12½-13½	13½-14½	12½-13½	broken	16½	12½	15½	14½-15½	13½	11½	12½-13½	broken	11½-12½	14½	13½-15½	14½-16½	13½
bighorn sheep	6^+ male (no. 1)	right	X	X	X	2½	X	X	X	4½	X	X	2½	3½	X	X	X	X	1½	2½	X	3½	X
	10^+ male (no. 12)	right	8½	8½	X	broken	7½	7½	X	broken	X	4½-5½	X	5½	8½	8½	X	4½	X	5½	X	7½	6½
mountain goats	4^+ male (no. 23)	right	X	X	X		X	X	X		2½	½	3½	1½	X	X	X	2½	½	2½	X	miss-ing	½
	14^+ male (no. 1)	right	X	X	X		X	10½-12½	X		X	miss-ing	11½	8½	7½	7½	X	missing					
domestic cattle	8 yr., 4 mo. male, (no. 1)	right	X	X	X	X	X	X	X	X	5½	X	X	X	X	X	X	3½	X	X	X	X	X
	7 yr., 4 mo. male (no. 2)	right	X	X	X	X	X	X	X	X	X	X	X	5½	X	X	X	X	1½	X	X	X	X

X = unreadable

or the most recently deposited cementum layer in most of the teeth, is opaque. In the teeth of bighorn sheep, bison, and mountain goats (the wild species), the opaque layers of cementum are wider than the translucent layers (Fig. 6 and 9). In the majority of domestic cattle teeth, however, there is no size difference between the opaque and translucent layers (Fig. 7). The annuli are also much thinner in relation to the total thickness of cementum compared to the annuli in the teeth of the wild species.

In the wild species, the opaque layers in the root cementum are sometimes made up of finer lamellae consisting of alternating opaque and translucent layers of cementum of equal thickness. These layers are reminiscent of the cementum layers found in the majority of cattle teeth.

In most cases there is a gradual decrease in the thickness of each succeeding annulus in the cementum of the wild species (Fig. 6). However, there is some variability in this regard, especially in the coronal cementum of the molars (Fig. 9). Also, as the size of the tooth increases from PM_2 to M_3 the annuli are correspondingly thicker. Incisiform teeth have the thinnest annuli.

In bison teeth, annuli are easily recognized because of the marked contrast between the opaque and translucent layers

of cementum, and because the layers are evenly deposited about the roots. In some incisiform teeth of bison, a thin translucent layer of cementum divides each opaque layer into two distinct thinner opaque layers. This condition was not observed in the smaller cementum annuli of the incisiform teeth of bighorn sheep, mountain goats, and cattle.

Additional observations of the cementum of bison teeth, from 17 lower jaws, indicate that although the number of annuli increases with age, the thickness of each annulus and thus the total thickness of the cementum of any one tooth varies between individuals of the same age. It was also found that the roots of all bison teeth, partially erupted, consist of a thin layer of dentine with little or no cementum. However, the crowns of all bison teeth, partially erupted, are well developed. At this stage the lingual and labial surfaces of the crowns of molariform teeth were covered with appreciable amounts of cementum.

In cattle teeth the root cementum is evenly deposited, but the annuli are too thin and too numerous to be counted accurately in the two specimens studied (Table 2). In a few cattle teeth, the root cementum consisted either of; (a) no annuli, in which case the cementum was opaque, or (b) annuli that resembled those found in the tooth cementum of the wild

species. These annuli were counted (Table 2).

Cementum annuli, in incisiform teeth of bighorn sheep and mountain goats, are difficult to count (Table 2) because they are crowded together in the thin cementum of these teeth. In the roots of the molariform teeth of bighorn sheep and mountain goats, the annuli are difficult to count (Table 2) because cementum deposition is irregular (Fig. 5). In older specimens, this irregular deposition of cementum produces rugose bosses on the roots.

In the majority of the roots of molariform teeth of the wild species, and in a few cattle teeth where the cementum annuli resemble the type found in the wild species, a portion of the cementum is divided into opaque and translucent layers of varying thicknesses (Fig. 4). These annuli did not seem to bear any relationship to the age of the animal. The presence of these "false annuli" in the root cementum makes it difficult to distinguish true annuli. Because of this, root cementum of molariform teeth of the wild species was abandoned for use in age-annuli comparisons.

Apart from the teeth of mountain goats, annuli in the coronal cementum are deposited evenly in vertical sheets on the crown surfaces of molar teeth. Each additional annulus of coronal cementum is shorter in length than the previous

one. Although this type of cementum is subjected to more loss and breakage than root cementum, because most of it is above the alveolar border of the jaw, the annuli can be easily distinguished, except in cattle teeth. False annuli are less prevalent in the coronal cementum of bison and bighorn sheep (Fig. 6 and 9).

In Table 2,the permanent teeth are put in the approximate order of their first appearance in the jaw by combining the tooth replacement methods of aging; bison (Fuller, 1959), bighorn sheep (Taber, 1963), and mountain goats (Brandborg, 1955). Apart from the variability in the number of annuli recorded for each tooth, the tooth most recently erupted has fewer annuli. Also the older specimens of each species have a greater number of annuli than the younger specimens. In these older specimens, as the annuli decrease in thickness and increase in number, it becomes more difficult to count the total number of annuli.

In bison, the number of cementum annuli of corresponding teeth from both rami of the same jaw is the same, although some variability is noticeable (Table 2). Also no difference in the appearance of the tooth cementum was noticed between male and female bison.

On the basis of the examination of the complete tooth

row, the root cementum of the canine (Fig. 8) and the coronal cementum of M_3 (Fig. 6) in bison, and the coronal cementum of M_3 (Fig. 9) and M^3 (the third upper molar) of bighorn sheep, were chosen as being most likely to be useful for age determination. The cementum of these teeth was chosen because; (a) the annuli could be seen easily, (b) false annuli were uncommon in the cementum, and (c) fewer annuli were observed in the cementum of these teeth, because they were the last teeth to erupt in the tooth row. Fewer annuli reduce the error in counting the total number.

There was some doubt as to whether the lingual cementum of M_3 of bison should be used for age-annuli comparisons since there was a variability in the number of annuli observed in the two specimens studied (Table 2). Therefore, the lingual cementum of either the left or right M_3 from each jaw was removed and sectioned from 31 lower jaws ranging from 1 year to 4½ years of age. The jaws were assigned an age by the method of Fuller (1959).

Fig. 10 indicates the results. In 1-year-olds the opaque layer of cementum does not cover the entire lingual and labial surfaces of the crown of M_3 and is thus considered to be less than half of an annulus. The third lower molar is not erupted at this time. In 2-year-olds killed in May, and 2½-

year-olds killed in November, the opaque layer covers the entire lingual and labial surfaces of the crown of M_3, and is thus, designated as half of an annulus. The third lower molar is beginning to erupt at this time. Therefore, if one annulus is added per year, beginning with half an annulus, the number of annuli for each specimen should all fall on either the upper (November specimens) or lower (May specimens) straight lines shown in Fig. 10. However, this is not the case because only four out of seven 3½-year-olds showed the expected number of cementum annuli and one 3-year-old had as many as 2½ cementum annuli in its lingual cementum. This type of variability continues in 4-and 4½-year-olds. It is also very difficult to count the total number of annuli, due to the presence of false annuli in the tooth cementum of these young specimens. Therefore the lingual cementum of M_3 was discarded as a method of age determination in bison.

In the young-adult and aged specimens of bison in Table 2, the cementum of C_1 contains 1½ annuli and 13½ annuli, respectively. If it is assumed that; (a) the first layer of cementum is not deposited on the tooth until it is erupted fully and its time of eruption is known, and (b) one annulus is equivalent to one annual increment of cementum, then the chronological age of the specimens can be determined.

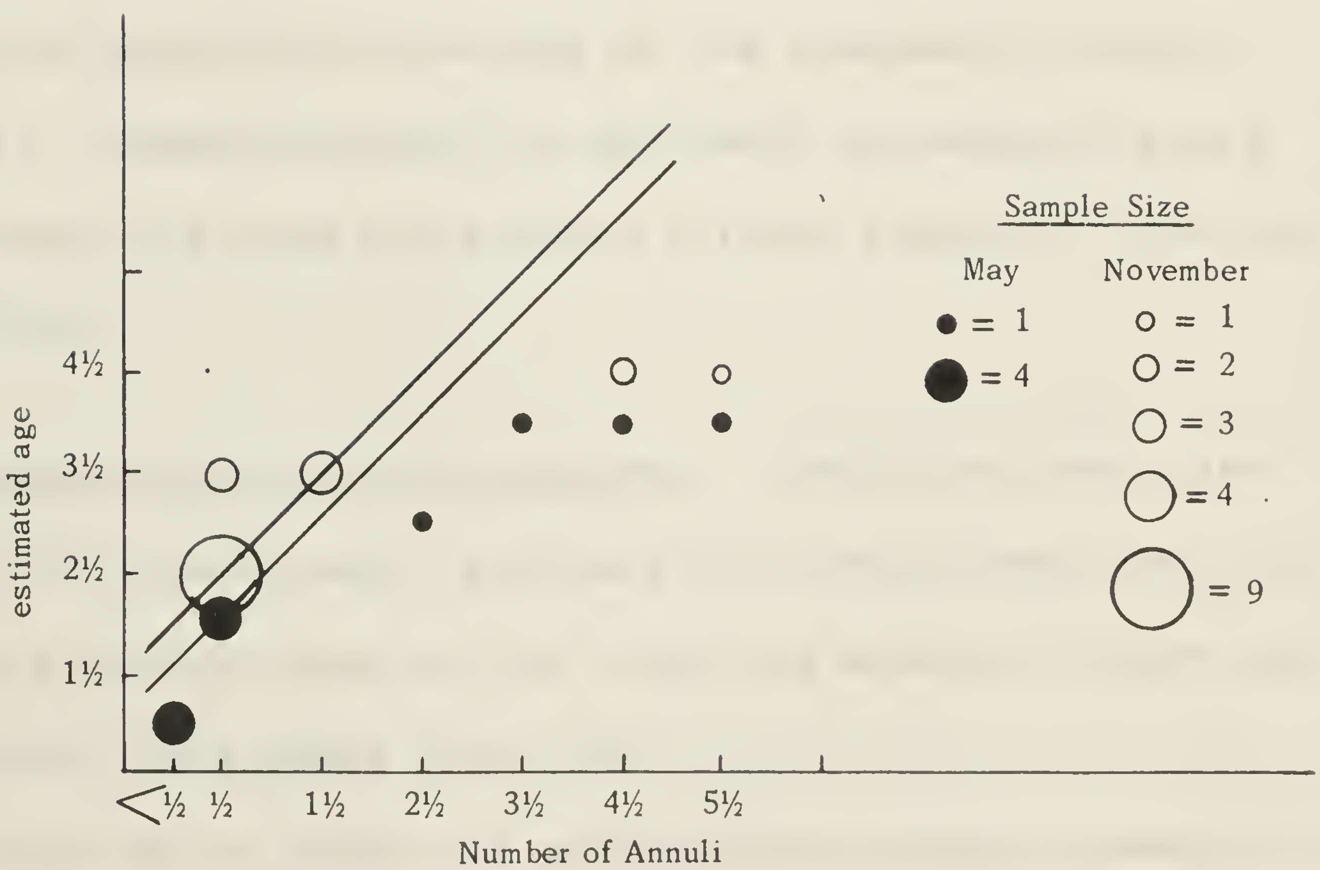

Figure 10. Annuli of the lingual cementum of M3 and estimated age of bison

In bison C_1 is erupted fully at 4 years of age (Fuller, 1959). On this basis, the young and old specimens are 5½ and 17½ years of age respectively. These ages agree with the broad-age categories as designated in Table 2.

If the same assumptions above are applied to the bighorn sheep specimens in Table 2, the ages of no. 1 and 12 are 6½ and 10½ years, respectively, because M_3 erupts at 3 years of age (Taber, 1963). The number of horn rings in these specimens is 6^+ and 10^+, respectively.

No particular tooth in cattle constantly exhibited annuli that agreed with the age of the specimens studied (Table 2). Cementum annuli in the teeth of mountain goats were present but were not studied further because of the lack of specimens.

Examination of horn sections. - Definite layers are found in the longitudinal sections of horns of ewes (Fig. 11). Also the internal rings of the horns are easier to count than the external horn rings (Fig. 11).

There are a number of similarities between cementum in the teeth of the wild species studied and the internal sections of horns of bighorn sheep ewes;

(a) The cone-within-a-cone construction found in the

root cementum of incisiform teeth is evident in the horns.

(b) With the exception of the first annulus, all subsequent annuli in horns decrease in thickness with age.

(c) Finer lamellae of alternating opaque and translucent layers were also prevalent in the opaque layers of horns.

(d) Each annulus in the horn sections is made up of one opaque and one translucent layer, although this condition is less evident than in the cementum annuli of teeth. There is one marked dissimilarity between the annuli in horn sections and annuli of tooth cementum. In tooth cementum, the most recently deposited annulus is the outermost one; in horn, the most recently deposited annulus is the innermost one.

Annuli and Tooth Wear in Bison

Canines were removed from 31 lower jaws ranging in age from 4 years to aged. The jaws were assigned an age by the method of Fuller (1959). From each lower jaw one canine was ground transversely, while the other was ground longitudinally, until the greatest number of annuli were seen. The count of annuli in one tooth thus served as a check on the other.

The greatest lingual and labial width of the worn biting surface of the canines was recorded to the nearest tenth

of a millimeter. The measurement included both the worn enamel and exposed dentine.

No tooth wear was present on the newly erupted canines of five specimens that were 4 or 4½ years old. The root cementum of these canines consisted of half of an annulus, or one opaque layer. Tooth wear occurred on the canines of specimens in the three additional age groups. A significant correlation ($r = 0.851$; $p = < 0.05$) was obtained between the number of cementum annuli and wear for each specimen of these age groups. Fig. 12 shows the linear relationship between wear and the number of complete annuli in the cementum of C_1.

The range in the number of annuli for each age group is shown in Table 3. Since the first opaque layer of cementum in C_1 is deposited when the animal reaches 4 years of age, a factor 4 is added to the number of annuli to establish the age ranges in Table 3.

Annuli and Known Age in Bison

The canines were prepared and their annuli observed from 19 known-age specimens. Thirteen of these lower jaws were from Elk Island Park and six were from the Wichita Refuge (Table 1). The known age of each specimen was compared with the number of annuli in C_1.

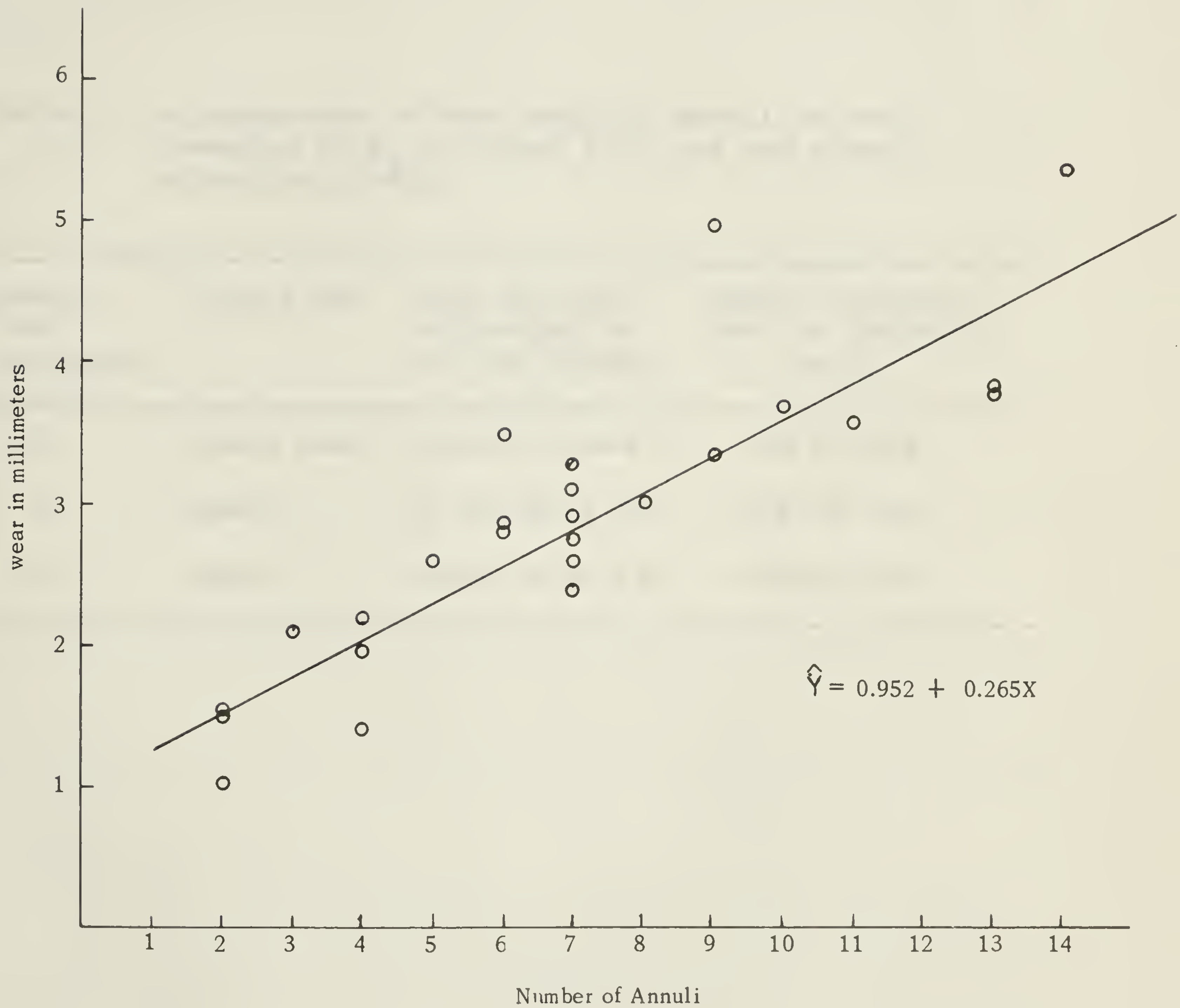

Figure 12. Wear and number of annuli in the cementum of C_1 in bison

Table 3. A comparison of the range of annuli in the cementum of C_1 of bison with the age groups of Fuller (1959).

Number of Specimens	Age group	Age in years according to Fuller (1959)	Number of annuli (+4) in cementum of C_1
14	young adult	mostly 5 and 6	6½ to 11½
6	adult	7 to 12 or 15	11½ to 15½
5	aged	over 12 to 15	13½ to 17½

If one annulus is added per year, beginning with half an annulus when the tooth erupts at 4 years of age, the points should all fall on the straight line shown in Fig. 13. The agreement with the expected result is good, although not perfect.

In the 10½-year-old (W.R. 10), the annuli in the cementum of C_1 were difficult to count because of a poor preparation. Therefore the root cementum of the third lower incisor (I_3) was used instead. Since I_3 erupts a year sooner than C_1 (Fuller, 1959) one additional annulus was expected and was, in fact, found.

In one 4½-year-old (W.R. 4x) the canines were just beginning to erupt and no cementum was evident on the roots. As described previously, the canines should be erupted fully at 4 years of age and contain half of an annulus. Tooth development might have been slower in this specimen or it might have been born late.

In the canine cementum of the two oldest specimens (14½ and 17½ years) the total number of annuli was one less than the expected number (Fig. 13). This was due to the difficulty of counting all the annuli in the older specimens, where the outermost layers become extremely thin.

Despite some variability, there is a highly significant

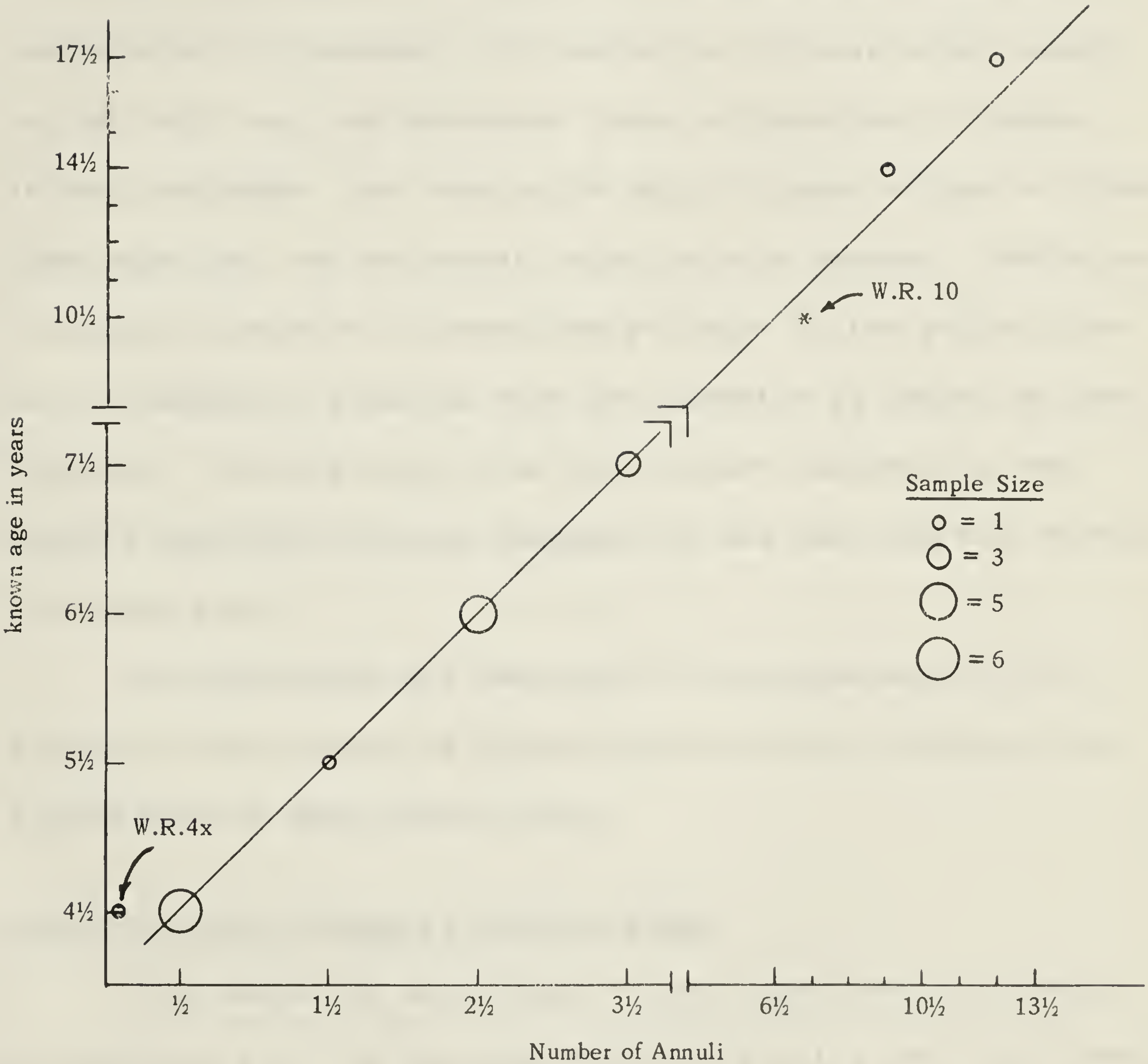

Figure 13. Annuli of the cementum of C_1 and known age in bison. * I_3 used instead of C_1. See text.

correlation between the number of annuli in the cementum of C_1 and the known age of bison ranging from 4½ to 17½ years of age ($X^2(19) = 0.75$; $p = < 0.05$).

In the cementum of C_1 of all the December specimens (as late as 17 December), including the 4½-year-olds, except for No. W.R. 4x, the outermost layer of cementum is opaque. In May specimens (as late as 26 May), 4 years of age or older (see page 29), the outermost layer is also opaque. Therefore, the opaque cementum is deposited at least in the period from May to December, assuming that the cementum is deposited continually. On this basis, the translucent cementum is deposited somewhere between December of one year and May of the following year.

No difference was detected in the appearance of the annuli in the canines of bison from the Wichita Refuge, Elk Island Park or Wood Buffalo Park.

Annuli and Horn Rings in Bighorn Sheep

The number of horn rings of each specimen was counted by biologists of the Canadian Wildlife Service and the Alberta Department of Fish and Game. Also for each specimen, the coronal cementum of either the left or right third molar was sectioned and the number of annuli recorded. Most of the

specimens consisted of upper or lower jaws with the exception of three rams and two ewes, in which the matching upper and lower jaws were present for each of these specimens.

The number of horn rings and cementum annuli were used in the following comparisons:

(a) In each of 11 rams, ranging from 1^+ to 3^+ horn rings, the number of horn rings was compared with the number of annuli and with age by tooth replacement (Taber, 1963).

(b) In each of 44 rams ranging from 2^+ to 14^+ horn rings, which includes six rams in (a), the number of cementum annuli was compared with the number of horn rings. In two rams (6^+ and 9^+) only, the upper and lower jaws were present which gave a total of 46 specimens (Fig. 14) for comparison.

(c) In two rams and three ewes a comparison was made between the number of annuli in M_3 and those of M^3 of each matching upper and lower jaw.

(d) In six ewes, the number of horn rings, both external and internal, was compared with the number of annuli.

The yearly age by tooth replacement, corresponds to the number of horn rings in rams with 1^+ to 3^+ horn rings. This suggests, at least in young rams, that 1^+ horn rings is equivalent to one year of age.

No coronal cementum was present on M_3 in the five rams

with 1^+ horn rings. The first sign of cementum was a thin opaque layer in the four rams having 2^+ horn rings (Fig. 14) and M_3 not erupted fully. This opaque layer became thicker but there was no translucent layer in the two rams with 3^+ horn rings. This thick opaque layer in the fully erupted M_3, was designated as half of an annulus. Thus in order to determine the age in years of the specimen, assuming that one annulus is deposited annually, a factor of 3 is added to the number of annuli present in the coronal cementum of M_3 and M^3. Variability in the number of annuli in specimens with 4^+ horn rings (Fig. 14) or more is due to the difficulty of recognizing them in the cementum.

If one annulus is added per horn ring, beginning with half an annulus when the tooth erupts at age 3, the points should all fall on the straight line shown in Fig. 14. The agreement with the expected result is good, although not perfect. In 11 specimens, an error of ± 1 annulus occurred and in 3 specimens an error of ± 2. Also, in all but four fall specimens, the outermost layer was opaque. All specimens were killed in the fall except J16, which was killed on May 27. Thus, based on the assumption that one annulus is equivalent to one annual increment of cementum, and that cementum is deposited continually, the opaque layers are deposited, at

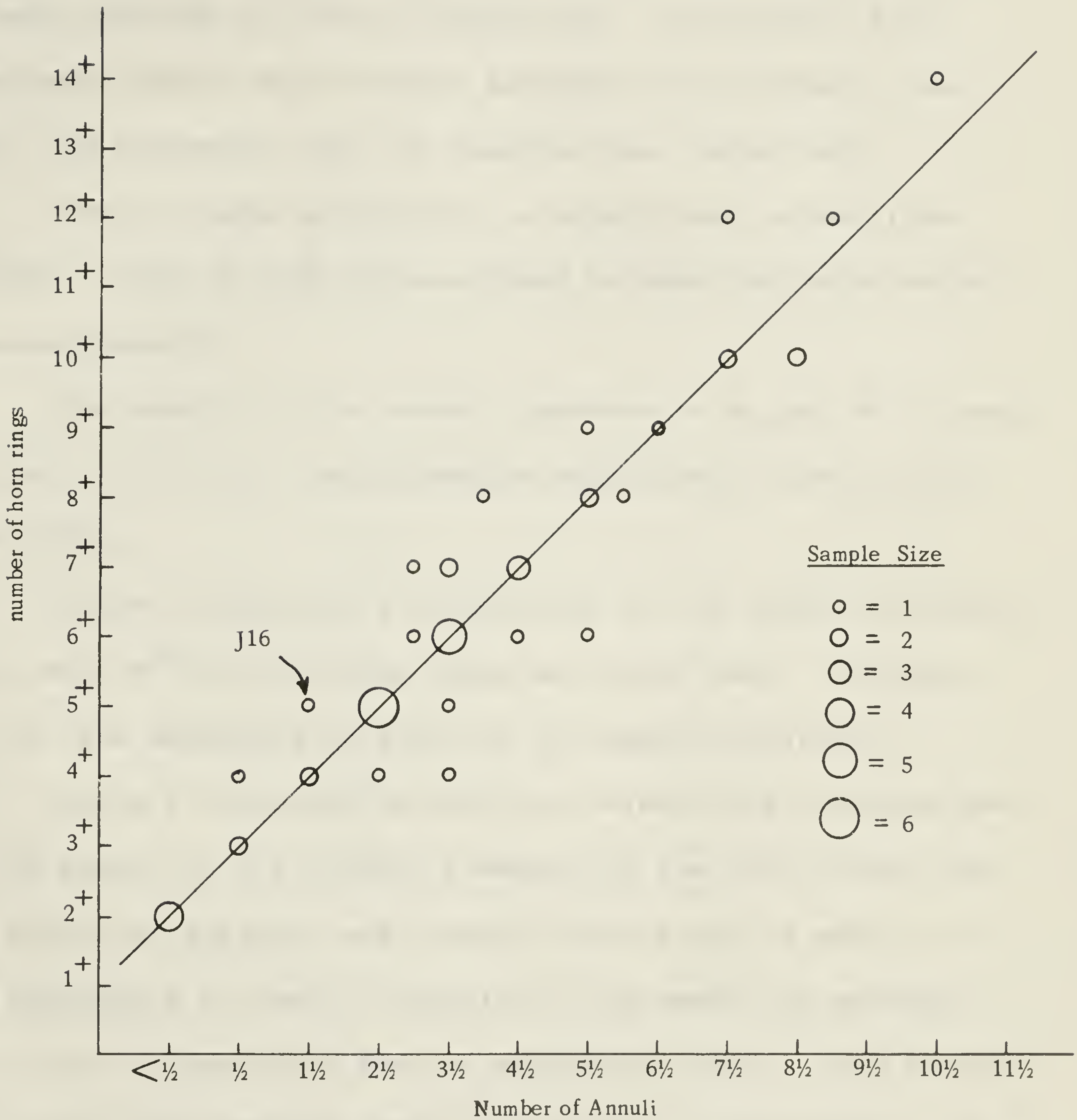

Figure 14. Annuli of the coronal cementum of M_3, and horn rings in bighorn sheep rams.

least from May to November (one specimen died as late as November 21), and the translucent layers somewhere between late November and May of the following year. In the four fall specimens, (which died between September and October), however, the outermost layer of cementum was translucent.

Despite some variability, a significant correlation ($X^2(46) = 7.95$; $p = < 0.05$) was found between the expected and observed results.

The annuli in the coronal cementum of M_3 and M^3 of rams with 9^+ to 14^+ horn rings, show up more clearly than do the horn rings.

Table 4 indicates a variability in the number of annuli of M_3 with M^3 from matching upper and lower jaws. In three out of five specimens an error of ± 1 annulus occurred.

Table 5 indicates variability between the observed number of annuli in the coronal cementum of the third molar and the number of external and internal horn rings in ewes. It was impossible to count, accurately, the number of external horn rings in ewes with four or more horn rings. The internal horn rings and cementum annuli were easily recognizable. If a factor 3 is added to the number of cementum annuli, the time at which the beginning of the first annulus is deposited in the third molars of rams, it is evident that the number of

Table 4. A comparison of the number of cementum annuli of M_3 with M^3 of matching upper and lower jaws from bighorn rams and ewes.

Jaw No.	Number of annuli	
	M_3	M^3
17	3½	3½
18	5½	6½
F4	1½	2½
F3	4½	3½
F1	6½	6½

F = female.

Table 5. A comparison of the number of cementum annuli in the third molar with the number of external and internal horn rings in bighorn ewes (date of collection unknown).

Jaw No.	Horn rings		Number of Observed Annuli
	External	Internal	
UF5	4^+	4^+	1½
F4	5^+	4^+	1½
UF6	6^+	7^+	3½
F3	6^+	7^+	4½
UF8	6^+	11^+	5½
F1	6^+	9^+	6½

U = upper jaw.

internal horn rings and cementum annuli correspond in four out of six specimens. It is believed that a technical error was responsible for the two discrepancies.

DISCUSSION

General Characteristics of Tooth Cementum and Horn Keratin

Location of tooth cementum. - "In human teeth, the cementum is thicker around the apex of all teeth and in the bifurcation of multirooted teeth than any other areas of the root" (Orban, 1957). This is in general agreement with all teeth used in this study and with teeth used in previous studies (Mitchell, 1963; Sergeant and Pimlott, 1959). Coronal cementum, and its relationship to age, however, has not been mentioned in previous studies on wild bovids and cervids and yet it is very characteristic of the bovid species. This type of cementum of herbivores, increases the occlusal surfaces of the molariform teeth and thus, offers more grinding surface (Gottlieb, 1942). The absence of coronal cementum in incisiform teeth relates to the function of these teeth, which are used mainly for cutting the food for subsequent grinding by the molariform teeth. Coronal cementum is negligible in species of North American Cervidae, as examination of museum specimens at the University of Alberta testifies.Thus, bovids have an advantage over cervids, in that coronal cementum, which increases in thickness with age, as determined

in this study, continually improves the functional ability of molariform teeth.

Function and cementum apposition. - The explanation for the local thickenings of root cementum has been based mainly on function. Bolden (1962), referring to human cementum, states that "normal tension stimulates the growth or apposition of the cementum. If continuous, normal tension may result in excessive cementum apposition . . ." Thus normal tension would be caused by the occlusal forces brought to bear on teeth opposing one another, as in the chewing process. Therefore, it seems likely that the areas of greatest cementum apposition receive the most tension.

Function conveniently explains the presence of thinner cementum on the incisiform roots compared to molariform root cementum in any one species, because incisiform teeth do very little grinding and therefore receive less pressure.

An irregular pattern of cementum on multirooted teeth of bighorn sheep and mountain goats, was characteristic. In older specimens of bighorn sheep and mountain goats. this irregular deposition of cementum developed into rugose bosses on the roots. The condition, found in the older

specimens, resembles that mentioned by Orban (1957) in relation to human teeth. It is called excementosis. He describes this condition as a cementum overgrowth not correlated with increased function of the tooth. The reasons for this condition are still in debate (Provenza, 1964). In the younger specimens, the cementum on the tooth roots of bighorn sheep and mountain goats resembles, what Orban (1957) terms, a cementum hypertrophy, where overgrowth of cementum improves the functional qualities of the cementum. This condition, he states, is frequently found in teeth which are exposed to great stress. The cementum forms projections increasing the surface area of the cementum for additional attachment of connective tissue fibres, which improves the suspensory apparatus of the tooth. Mitchell (1963), found that teeth of red deer, showing abnormal wear, had correspondingly thicker deposits. This condition was not observed on the molariform roots of cattle and bison teeth.

Kronfeld (1938) and others in human dental research, give evidence that function is not necessarily a stimulating factor in cementum apposition. Some of the results of the present study support this opinion. For example, it was found in the wild species that the thickness of each annulus

and thus, the total thickness of cementum, increased with the size of the tooth, from PM_2 to M_3, regardless of which tooth received the greatest wear and thus the greatest occlusal force.

In the teeth of mountain goats and bighorn sheep, the cementum was thicker at the root apices than at the bifurcation of multirooted teeth, whereas the converse was true for bison and cattle teeth. Since occlusal forces would be expected to be similar in all these species, this suggests that tension alone is not a sufficient explanation. Furthermore, in bison teeth there is considerable variability in the thickness of cementum between individuals of the same age which suggests that heredity, food preference or environmental conditions may affect cementum deposition.

<u>Deposition of the first layer of cementum</u>. - Except for a few cattle teeth, cementum in all teeth consisted of alternate opaque and translucent layers. This is in agreement with previous work done on terrestrial and marine mammals. Sergeant and Pimlott (1959) suggest that the beginning of the first opaque layer of cementum is deposited on the roots of the first incisor of moose about the time of birth, before the tooth is erupted fully. They found that this first opaque layer had a speckled appearance not seen in

later layers. Low and Cowan (1963) also found a more cellular opaque layer on the roots of the newly erupted first incisor of mule deer. In the present study, the first layer of cementum deposited was opaque, but was not noticeably different from later opaque layers. There was very little or no cementum present on the roots of bison teeth not erupted fully. Patten (1958), referring to human teeth, supports this finding by stating that "no cementum is found until the tooth has acquired nearly its full growth and approximately its definitive position in the jaw . . ." However, from a survey of bison and bighorn sheep teeth it was found that the coronal cementum of the third molar was deposited 2 years before its complete eruption, and yet no appreciable difference in appearance was noticed between this opaque layer and subsequent opaque layers.

Deposition of cementum in partially erupted teeth. - According to Orban (1957), in a developing tooth the cells of a portion of the epithelial sheath that cover the crown differentiate to form enamel, whereas those that cover the root induce the formation of dentine. The epithelial sheath also separates the periodontal connective tissue, which contains cementoblast cells responsible for cementum formation, from the tooth surface. When the epithelium degenerates, the periodontal connective

tissue is allowed to come in contact with the surface of the tooth and thus cementum is deposited in this area. In partially erupted teeth of bison and bighorn sheep, this process of degeneration must occur at the crown because cementum is present only on the crown and not the root. At this stage the crown is well developed, but the root consists only of a thin layer of dentine. In most cases, cementum is not noticeable on the root until the tooth becomes surrounded by a well-formed socket and comes into occlusion.

Because the cementoblast cells of the periodontal connective tissue lining the alveolus of the tooth,are responsible for cementum deposition, it is important that, when transverse sections of teeth are desired to expose the full complement of cementum annuli, the cut be made below the alveolar border.

Characteristics of finer lamellae. - In the wild species of this study the opaque layers of cementum were considerably wider than the translucent layers. This in in agreement with others working in this field. Most wide opaque layers, in the root cementum of the wild species and in horn keratin of bighorn ewes, were made up of finer lamellae of alternating opaque and translucent layers. This same

condition was found by Low and Cowan (1963) in the tooth cementum of deer, and Nishiwaki _et al_. (1958) in tooth dentine of the sperm whale (_Physeter catodon_). Low and Cowan (1963) speculated that these lamellae result from fluctuations in food intake or from deep-seated rhythms in growth. Their morphology certainly suggests some form of cyclic activity. There is no evidence of these fine lamellae in the translucent layers of tooth cementum or horn keratin.

Characteristics of the "rut line". - Low and Cowan (1963) mentioned the occurrence of what they term a "rut line" in the opaque cementum of teeth of deer. This "rut line" consisted of translucent cementum and it was suggested that its presence was the product of an interference with normal metabolism during the rut. Similar translucent layers were found in the cementum of some incisiform teeth of bison and served to separate the opaque layers into two distinct parts. This condition was found in both sexes. It is suggested therefore, that a cyclic change in the metabolism, as in the rut, occurs in both sexes.

Presence of false annuli. - False annuli were present in the tooth cementum of the wild species in this study. Their presence suggests that the causative factors

for such annuli are not seasonal, but sporadic. Therefore, it is suggested that the normal deposition of cementum is disrupted due to an abnormal physiological condition of the animal.

Some cattle teeth also consisted of opaque cementum with no layers, or cementum annuli similar to those of the wild species. Again, it is suggested that a disturbance in the normal physiological condition of the animal is responsible for the observed phenomena.

Seasonal Changes in Annuli

The results from the present study tend to support, in part, that cementum of teeth is subject to seasonal change. As Sergeant and Pimlott (1959) put it "it seems likely that opaque material is laid down in summer and fall, translucent cement in winter and spring".

In the present study the opaque layers in the cementum of C_1 of bison, were deposited during May to December and the translucent layers were deposited somewhere between December of one year and May of the following year. Mitchell's (1963) findings agree approximately, with the above results, because the outermost layer or more recently deposited cementum in red deer shot from May to December was opaque, and the most recently deposited cementum of red deer shot from January to

April was translucent.

The opaque layers of cementum of the crowns of the third molars of bighorn rams, were deposited between May and November, and the translucent layers sometime between November and May of the following year. Taylor (1962) found with known dates of death of bighorn rams in Montana, that horn growth began in mid-April and cessation of growth occurred in October. Therefore, it seems that the periods of growth and cessation of growth of horns, correspond approximately to the conditions seen in the cementum annuli. That is, the thick opaque layers seen in horn sections of bighorn ewes, and in the tooth cementum of the wild species, represent the growing period, and the thin translucent layers represent a cessation of growth. Taylor (1962) also found that horn growth ceased if rams became ill, which could occur at any time during the growth period. It seems reasonable to assume that a number of reactions to environmental stimuli could cause the cessation of growth in tooth cementum and horn keratin. Therefore, this cessation of growth in cementum could result in an earlier formed translucent layer than normal which was, in fact, found in four rams collected in the fall.

From a review of previous literature, and results obtained from the present study, two causes for seasonal changes

in cementum are suggested:

(a) Feeding habits, combined with nutritional conditions.

(b) Photoperiodism.

In this study, as in previous studies, the opaque layers consist of a greater concentration of cementocytes and, in the wild species, are wider than the translucent layers which contain very few cementocytes. Cementoblasts are those cells which form cementum matrix (Provenza, 1964). These cells, which become entrapped in their own matrix, are known as cementocytes (ibid.). Provenza (1964), referring to human teeth, points out that "when more acute stimulations which require rapid deposition of cementum matrix are experienced, the cementocytes are more numerous per unit area." He further states that this rapid deposition results in wider layers of cementum. The translucent layers of cementum represent resting lines and appear to be similar to the resting lines in bone (McMurchy , pers. comm.). In bone the resting line delimits the area of completion and renewal of continual bone deposition (Provenza, 1964). Because calcification of the matrix formed by the cementoblast cells, occurs as part of cementum deposition, it is suggested that the area of greatest cementocyte frequency (the opaque layers) is more calcified,

whereas the translucent layers, representing the resting lines with little cementum deposition, are less calcified. This suggestion is supported by the fact that the opaque layers in the cementum pads of bison teeth are proportionally more eroded by decalcifying agents than the translucent layers. Therefore it seems likely that the opaque layers are more mineralized than the translucent layers.

With the above characteristics of opaque and translucent cementum in mind, it is suggested that opaque cementum, representing continual deposition and a greater mineral content than the translucent cementum, could be due to the continual stimulation received from constant chewing during the late spring, summer and fall, of food that is readily available. The translucent cementum, representing almost complete cessation of cementum deposition and having a lower mineral content than the opaque cementum, could be due to the reduced amount of stimulation received from less chewing during the winter months of food that is less available. Tooth function as a part of the feeding habit, readily explains why the translucent layers of cementum in teeth do not occur until the teeth have erupted fully and have come into wear. That is, the first translucent layer appears during the time at which food is less available. However, tooth function as

part of the feeding habit does not explain the presence of appreciable amounts of coronal cementum on the third molars 2 years prior to their full eruption.

If nutritional conditions and feeding habits, as suggested causes for seasonal changes in cementum, remained relatively constant throughout the year, there should be practically no seasonal effect on tooth cementum. As the results indicate, the tooth cementum of cattle showed no seasonal effect.

Fisher (1954) found that unlike the harp seal (*Phoca groenlandica*), the harbour seal (*Phoca vitulina*) does not show well-defined annuli in the dentine. He attributes this difference to the fact that unlike the harp seal, the harbour seal does not undergo starvation periods and feeds throughout the whelping, mating, and moulting period. This presents further evidence that feeding habits combined with nutritional conditions, may be a factor responsible for seasonal changes in tooth tissue.

The present study presents conflicting evidence which suggests that feeding habits combined with nutritional conditions are not entirely responsible for the seasonal changes in cementum. There was no noticeable difference in the appearance of cementum annuli in the canines of bison from the

Wichita Refuge, Elk Island Park, and Wood Buffalo Park. Novakowski (1964) found that the nutrition of bison in Wood Buffalo Park may be adversely affected by deep snow (the mean depth is 4 feet) and a decline in the nutritive value of the forage (6 to 8% protein) in the winter. Conversely, the Wichita bison were on good range throughout the year (Howard, pers. comm.) and did not have to contend with snow cover. Despite the contrast between the two environments, there was no difference in appearance of cementum annuli in the lower canines of bison from the two different areas. However, Van Nostrand and Stephanson (1964) found with beaver, that regional differences contributed to differences in the appearance of cementum.

French (1960) speaking of white-tailed bucks (Odocoileus virginianus) said that "decreased food consumption and weight losses occurred regularly each fall at the time of the rut and also in winter despite the availability of unlimited amounts of high quality feed. These changes were followed by a cyclic increase in food intake and weight starting in April and reaching a maximum just prior to the rutting season in September." Also, French (1960) found in white-tailed bucks, that the severe winter decrease in food

consumption and weight could be lessened by increasing the amount of light beyond that which is usually received during the winter months. Wood, Cowan, and Nordan (1962) also found with deer (Odocoileus hemionus), that a restriction of food intake occurred during fall and winter even when food was plentiful. Low and Cowan (1963) state that "this behavior may rest on cyclic hormonal changes or other related photoperiodic responses." However, evidence is lacking for this type of cyclic behavior of the wild species used in this study. Therefore, it is suggested that the seasonal changes observed in tooth cementum of the wild species, including the horn keratin of bighorn ewes, may be due to seasonal differences in feeding habits and nutritional conditions (quality and quantity of food), or photoperiodism, or both.

Chronology of Tooth Cementum and Horn Keratin

The results of the examination of the complete tooth row of bison, bighorn sheep, and mountain goats, suggest the probability that cementum is continuously formed throughout the life of the tooth by deposition of new layers of cementum. This view is supported by Provenza (1964), and others in the field of human dentistry, and also by those working on

wild bovids and cervids (Mitchell, 1963; Low and Cowan, 1963; Sergeant and Pimlott, 1959; and Novakowski, 1964). The similarities of tooth cementum and keratin in horn sections of bighorn ewes, also suggest that keratin is deposited continually.

Annuli and tooth wear. - It was found in the present study that with an increase in tooth wear, which is an indication of age, there was an increase in the number of cementum annuli in the lower canines of bison. Thus, as each annulus is deposited, the tooth is pushed upwards to compensate for the wear received on its surface. Novakowski (1964) also found a good correlation in bison between wear and the number of annuli present in the root cementum of PM_4. He suggested that relating the number of annuli to incisor wear shows promise as a technique for aging large samples on autopsy. The same suggestion can be applied to wear and cementum annuli of C_1 of bison in the present study.

The method of aging to date, involves putting bison, that are older than 4 years of age, into three broad age groups based on tooth wear (Fuller, 1959). Fuller (1959) believed that the "young adult" group contained mostly 5-and 6-year-olds and that the division between adults and aged probably came at about 12 or 15 years of age. However, the

present study shows that the age range of young adults, based on the number of annuli in the cementum of C_1, was 6½ to 11½ years. The division between adults and aged more or less corresponds to the estimate of Fuller (1959). Sergeant and Pimlott (1959), and Low and Cowan (1963), also found that as tooth wear increased so did the number of cementum annuli. Based on their results, they found that the variation in ages, determined by tooth wear and compared with cementum layers, was so great, that doubt was thrown on the ranges of the age classes based on wear.

Annuli and increasing age. - Sergeant and Pimlott (1959) found that in older moose the later growth layers of cementum are thinner than the earlier ones. Novakowski (1964) found in bison that layers of cementum become progressively thinner with advancing age. This same phenomenon was found in dentine (McLaren, 1958, and others). Mitchell (1963), however, stated that teeth from younger red deer have thinner depositions of cementum than older deer.

In the present study, each succeeding annulus of cementum became progressively thinner in most of the teeth of the wild species, although some variability was noticed. Usually this progression was only noticeable when the first few annuli

were compared to later annuli in older specimens. This same decrease in thickness of each succeeding annulus was noticed in the keratin of horn sections of bighorn ewes, with the exception of the first annulus. The first annulus, which probably represents the first years growth, is not thicker than the second one, because the horns in the first 4 or 5 months of a ewe's life, are just beginning to develop, and project about one inch from the head (Cowan, 1940). After this period the horns develop rapidly for the next 4 years (*ibid.*).

Hewer (1960) found in the canine cementum of grey seals (*Halichoerus gypus*), that the earlier rings were broad and suggests that these broader rings are laid down before sexual maturity. Also Mundy and Fuller (1964) found that the first five annuli in the cementum of M_3 from grizzly bears (*Ursus* spp.) were clearly wider than those that followed. They suggested that the marked changes in the rate of deposition may prove to correlate with the onset of sexual maturity. In the wild species studied, however, the thickness of each cementum annulus, including the internal horn annuli of ewes, gradually decrease, so that no marked transition from thick to thin annuli is observed. Therefore it is difficult to determine which annulus would represent the onset of

sexual maturity. It is suggested that growth, which becomes slower with advancing age, is responsible for the decrease in thickness of each annulus with age.

The cementum annuli of some teeth of the wild species, however, did not show a continual decrease in thickness of each annulus but they remained relatively constant or varied in thickness. These differences could be due to some physiological change in the animal, from one year to the next.

Annuli and known age. - The number of cementum annuli in known-age bison showed that one annulus is equivalent to one year of age. This evidence is supported by previous workers in this field, especially by the work of Low and Cowan (1963) who used a representative sample of known-age jaws of mule deer.

A significant correlation was obtained between the number of horn rings and cementum annuli in the coronal cementum of the third molar, in bighorn rams. Nothing could be found in the literature with reference to a comparison of horn annuli to known-age of bighorn rams. However, Taylor (1962) gave substantial evidence that one horn annulus is equivalent to one year of age based on animals collected at different times of the year. This fact is also supported in the

present study, because the age in years of young rams, as determined by tooth replacement, corresponds to the number of horn rings for each specimen. Based on the above evidence therefore, it is probable that one cementum annulus is equivalent to one horn annulus, which is in turn equivalent to one year of age.

<u>Annuli as an Aging Criterion</u>

In the practical application of using the root cementum of C_1 for aging bison, older specimens may be over or under aged by $\pm$ 1 or 2 years. Probably, this variability is due to imperfect preparations of the teeth, or by a misinterpretation of the observed criteria. Accuracy of the method will improve with experience.

Evidence in this study and in previous studies, tends to support the contention that one horn ring is equal to one year of age. If this is so, then the accuracy of aging bighorn rams, by counting the number of annuli in the coronal cementum, and comparing them with the number of horn rings, is $\pm$ 2 years. It is believed, that an error of $\pm$ 2 years, is due to; (a) an error in counting the annuli, or (b) a loss during cutting, of part of the coronal cementum in which the annuli were probably most easily recognized.

Aging bighorn rams in this study by counting their horn rings is adequate to approximately 9^+ horn rings. In rams with 9^+ horn rings or more, the corresponding cementum annuli are easier to identify than the horn rings, which are very close together in these older specimens. Because of lack of known-age material, it could not be determined whether horn rings or cementum annuli would give the most accurate estimate of age in these older specimens.

In bighorn ewes the number of cementum annuli corresponded to the number of internal horn rings in four out of six specimens. Although this is not conclusive, because of lack of a representative sample, it is suggested that for aging ewes, both the coronal cementum and horns should be sectioned. Thus, one could act as a check on the other, with regard to the total number of annuli.

SUMMARY

1. Annuli in tooth cementum were studied in three wild species (bison, bighorn sheep, and mountain goats), and one domestic species (cattle).

2. Cementum annuli were observed adequately in; (a) thick sections of teeth of the wild species, in alcohol and under reflected light, and (b) thin sections of teeth of domestic cattle in transmitted light.

3. Differences in the location of cementum on the teeth were found. Function was discussed as a possibility for these differences.

4. There were marked similarities in the appearance of tooth cementum of the wild species and horn keratin of bighorn ewes.

5. The root cementum of the lower canines of bison and the coronal cementum of the third molars of bighorn sheep were chosen for age-annuli comparisons.

6. A significant correlation was found between cementum annuli and tooth wear in bison.

7. A comparison was made between the age of bison by tooth wear (Fuller, 1959) and age by the number of cementum annuli. Variation in ages determined by tooth wear and

compared with cementum annuli, was so great, that doubt was thrown on the ranges of the age classes based on wear.

8. It was suggested that the decrease in thickness of annuli with age in the wild species studied was due to growth, which decreases with advancing age.

9. A significant correlation was found between; (a) the cementum annuli and the known age of bison, and (b) the number of annuli and horn rings in bighorn sheep rams. Also a correlation was found between cementum annuli and internal horn rings of bighorn ewes.

10. Tooth cementum of bison and bighorn rams showed a seasonal change. Reasons for this change are discussed.

11. Bison up to 10½ years old were aged accurately to within a year by counting the number of annuli in the root cementum of the lower canine. The two older specimens were aged to within ± 1 year.

12. Bighorn sheep rams were aged to within ± 2 years by counting the number of annuli in the coronal cementum of the third molar. It was suggested that both the internal rings of horns, and annuli in the coronal cementum of the third molar should be counted to determine the age in bighorn ewes.

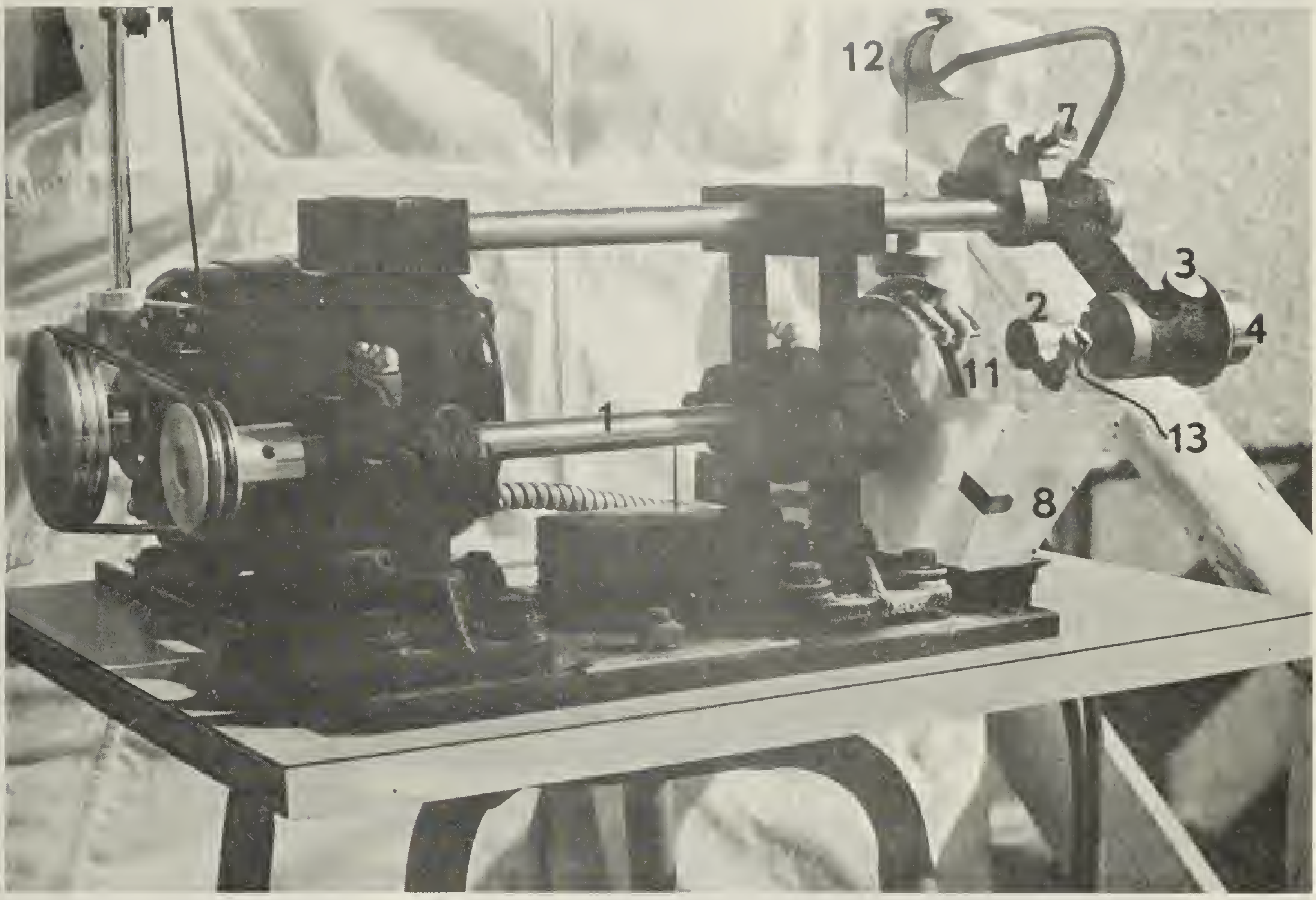

Figure 1. The Niclas Bone and Tooth Cutting Machine.

1. Cutter support shaft.
2. Specimen holder.
3. Micrometer lock screw.
4. Micrometer.
5. Cutter guard.
6. Water inlet (for cooling of specimen while cutting).
7. Depth of cut adjustment screw.
8. Splash guard surrounding cutter.
9. Micrometer bracket rest screw.
10. Micrometer bracket.
11. Cutter.
12. Balance weight.
13. Locking screw.

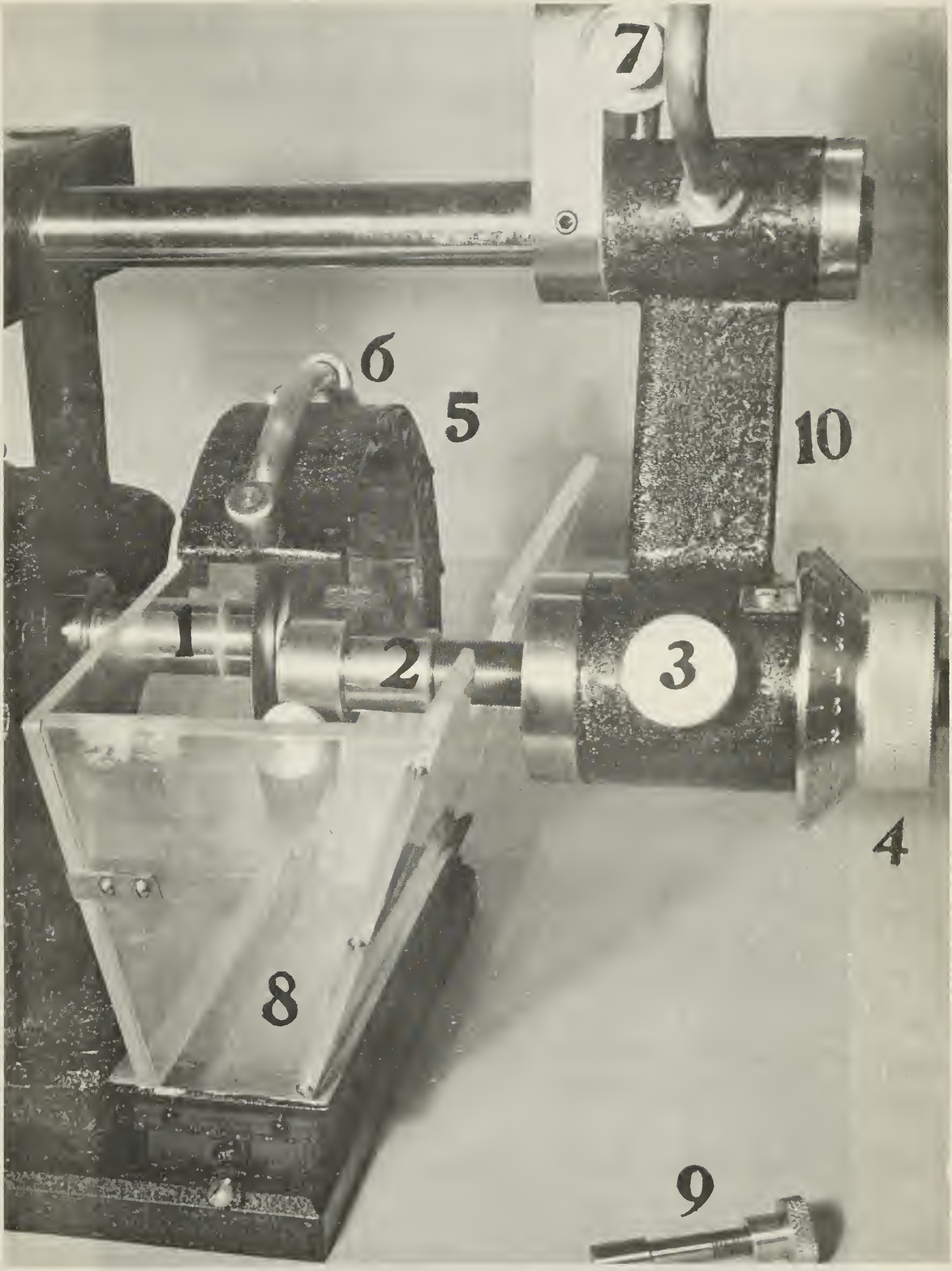

Figure 2. A close-up of the cutting area of the Niclas Bone and Tooth Cutting Machine. Explanation of numbers is given in Fig. 1.

Figure 3. Side view of bison dentary with part of the bone removed. The arrow points to coronal cementum.

Figure 4. Thick longitudinal section showing false annuli (F) in the cementum pad of M_3 of bison (No. 741).

Figure 5. Thin longitudinal section of the roots showing irregular deposition of cementum on M_2 of a mountain goat (No. 1). C, cementum; D, dentine.

Figure 6. Thin transverse section of the lingual cementum of M_3 of a bison (No. 741).

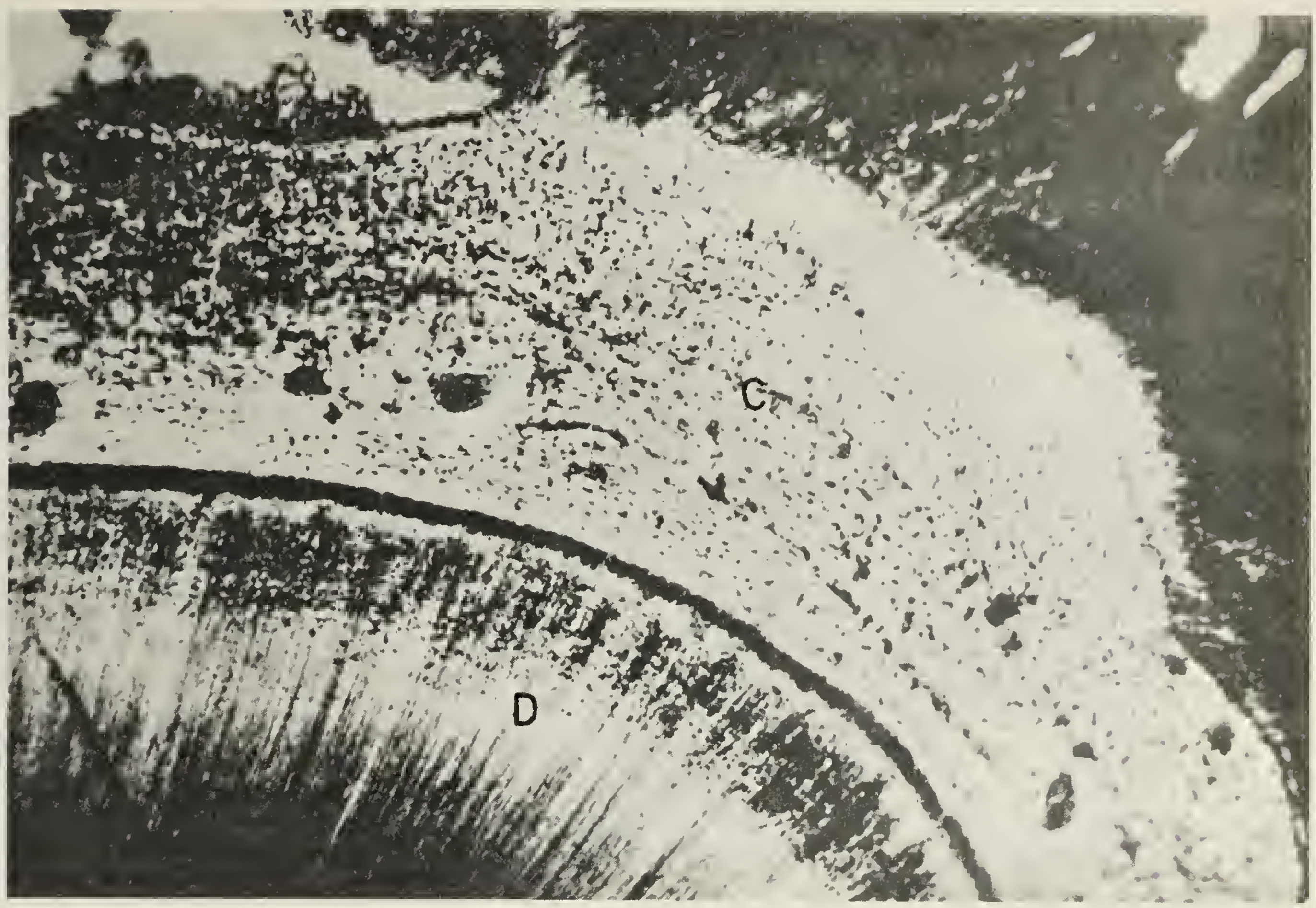

Figure 7. Thin transverse section of the root cementum of PM_3 of domestic cattle (No. 23) (x40).
C, cementum; D, dentine.

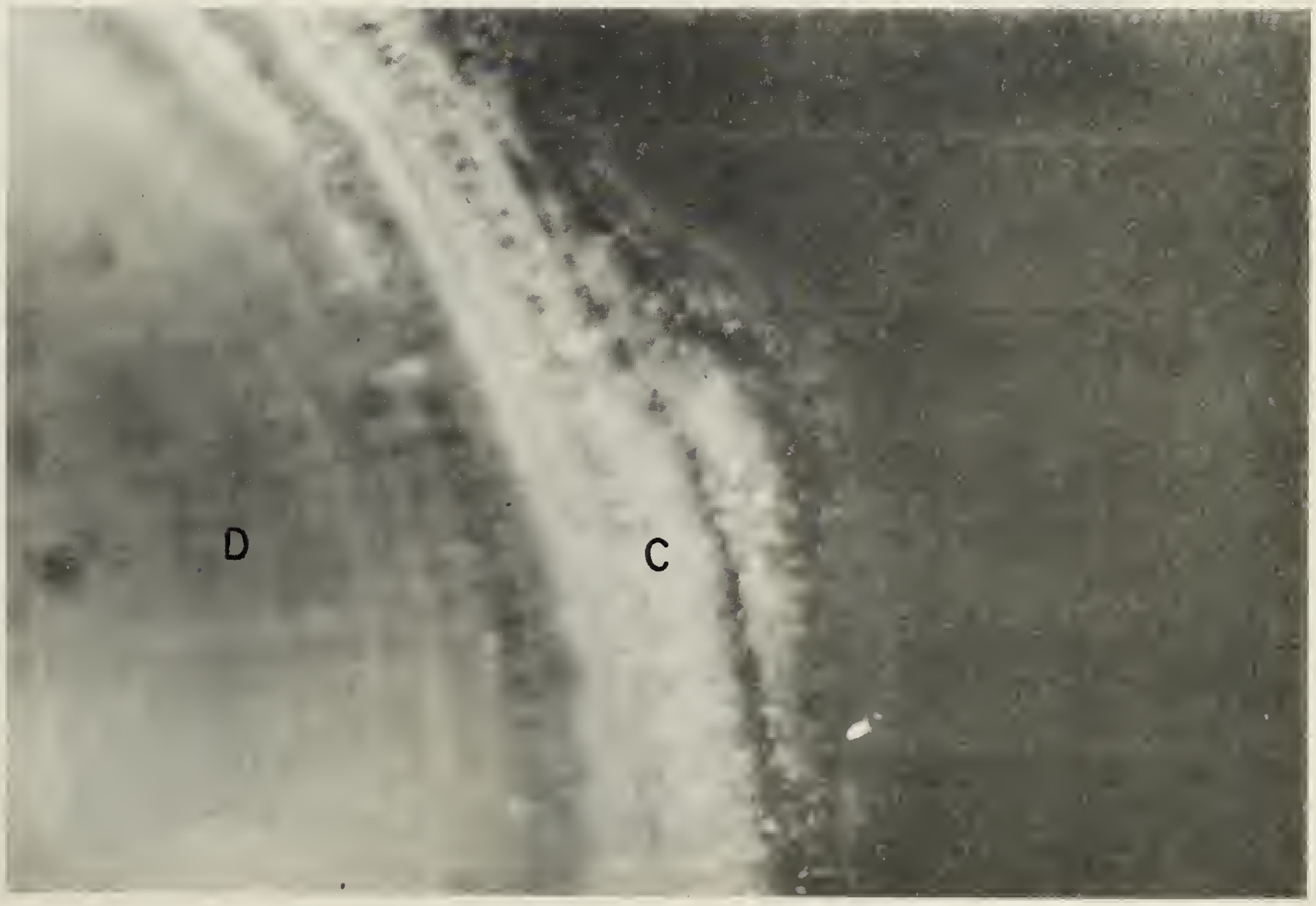

Figure 8. Thick transverse section of the root cementum of C_1 of bison (No. W.R. 7) (x16).
C, cementum; D, dentine.

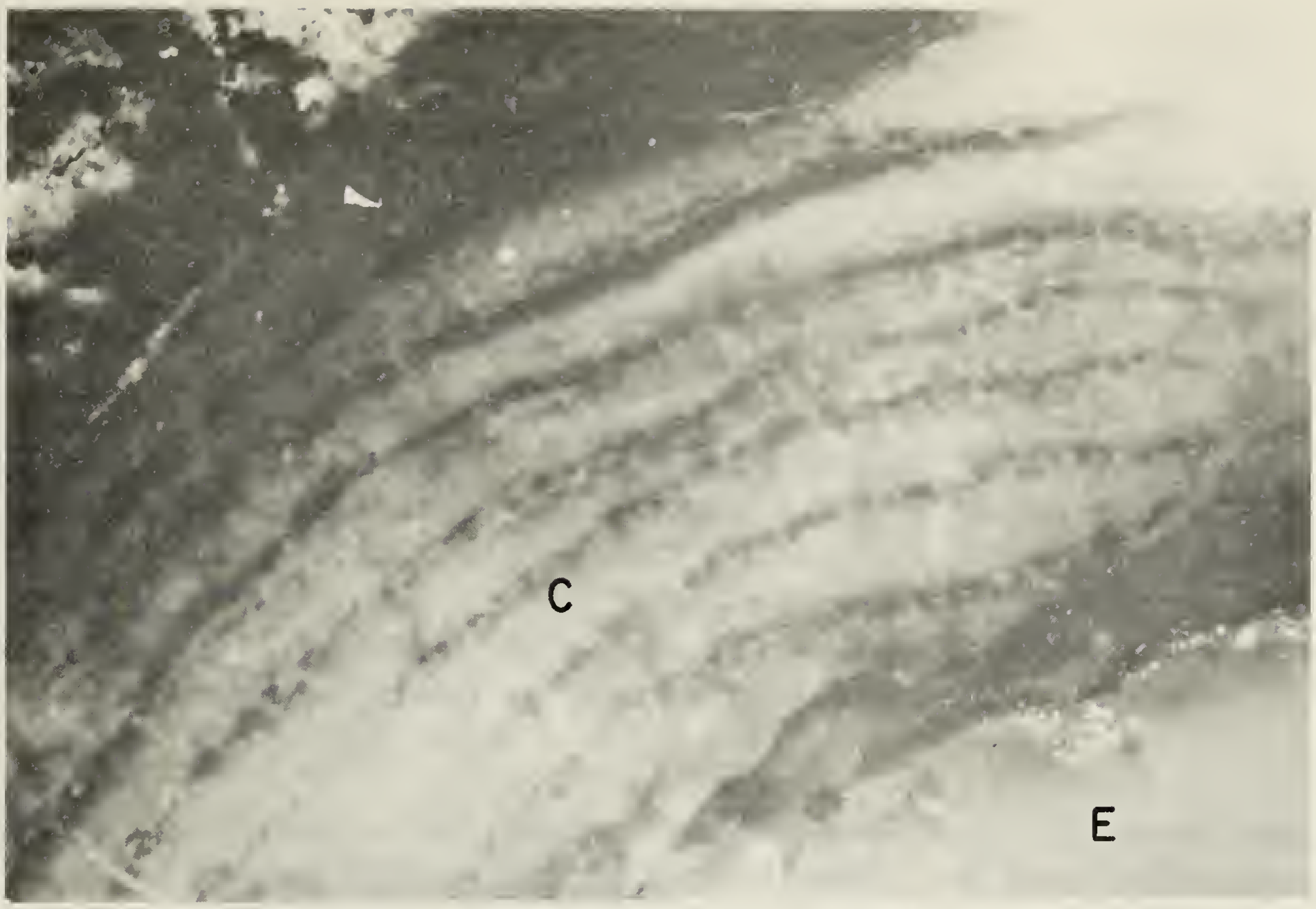

Figure 9. Thick transverse section of the coronal cementum of M_3 of a bighorn ram (No. J12) (x16).
C, cementum; E, enamel.

Figure 11. External and internal horn rings of a bighorn ewe (No. F1).

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